REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

PLEASE DO NOT RETURN YOUR FORM TO THE ABOVE ADDRESS.

1. REPORT DATE *(DD-MM-YYYY)*	2. REPORT TYPE	3. DATES COVERED *(From - To)*
5-9-2014	Final Report	January 2009-May 2014

4. TITLE AND SUBTITLE

The Physical Properties of A lavage mixture of pulmonary surfactant, perfluorodecaline, and methylprednisolone (perfectant lavage)

5a. CONTRACT NUMBER

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

Timothy F. Haley, MD, LTC, MC

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

Timothy F. Haley, MD, LTC, MC, USA
Division Surgeon
Division West, First Army
Bldg 410, Room 253D

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)

Nereyda Sevilla, MPH, CAsP, PMP
Office of the Air Force Surgeon General
SG5I, Research and Acquisitions
7700 Arlington Blvd, Ste 5164
Falls Church, VA 22042-5164

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION/AVAILABILITY STATEMENT

DISTRIBUTION A. Approved for public release: distribution unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

Purpose: To characterize the physical properties of a lavage mixture of pulmonary surfactant, perfluorocarbon and methylprednisolone.

Background: Perfluorocarbons (PFCs) are compounds derived from hydrocarbons by the substitution of hydrogen atoms with fluorine atoms. Perfluorocarbon liquids are colorless, odorless and biologically inert. They are highly dense, due to their molecular weight. Their low intermolecular forces give the liquids low viscosities (compared to liquids of similar boiling points), low surface tension and low heats of vaporization. PFC liquids are immiscible in most organic solvents, but are avid carriers of oxygen. The ability of PFC liquids to disperse readily throughout the lungs and allow for free gas exchange at the aveolar-capilary interface (allowing subjects to "breath") make these liquids ideal for delivering pharmaceutical agents to the lung and for whole lung lavage in certain disease states

15. SUBJECT TERMS

Perfluorocarbons, fluorine atoms, viscosities, aveolar-capilary interface, methylprednisolone

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			Timothy F. Haley, MD, MC
U	U	U	UU		19b. TELEPHONE NUMBER *(Include area code)* 210-382-7522

FINAL TECHNICAL REPORT
THE PHYSICAL PROPERTIES OF A LAVAGE MIXTURE OF PULMONARY SURFACTANT, PERFLUORODECALINE AND METHYLPREDNISOLONE (PERFACTANT LAVAGE)

INVESTIGATOR:

Timothy F. Haley, MD, LTC, MC, USA
Division Surgeon
Division West, First Army
Bldg 410, Room 253D
761st Tank battalion Avenue
Fort Hood, TX 76544
(210) 382-7522
timothy.f.haley.mil@mail.mil

BACKGROUND:

Perfluorocarbons (PFCs) are compounds derived from hydrocarbons by the substitution of hydrogen atoms with fluorine atoms (figure 1). Perfluorocarbon liquids are colorless, odorless and biologically inert. They are highly dense, due to their high molecular weight. Their low intermolecular forces give the liquids low viscosities (compared to liquids of similar boiling points), low surface tension and low heats of vaporization. PFC liquids are immiscible in most organic solvents, but are avid carriers of oxygen. The ability of PFC liquids to disperse readily throughout the lungs and allow for free gas exchange at the aveolar-capilary interface (allowing subjects to "breath") make these liquids ideal for delivering pharmaceutical agents to the lung and for whole lung lavage in certain disease states.

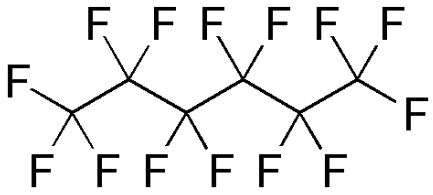


Figure 1

Pulmonary surfactant is a biological agent formed by type II alveolar cells within the alveolus of the lungs. The function of pulmonary surfactant is to reduce the surface tension within alveoli resulting in improved lung function. Active pulmonary surfactant is essential for alveolar stability, compliance, and gas exchange. The absence of a normal surfactant pool results in collapsed lung segments and contributes to Acute Respiratory Distress Syndrome (ARDS).

ARDS is characterized by acute inflammation of the lung parenchyma. The acute inflammatory-phase involves massive recruitment of neutrophils, and the systemic liberation of various cytokines. ARDS interferes with normal surfactant production and function and has a mortality rate of 30%.

Easa et al has described a method for the administration of surfactant in a large volume of liquid. Once the free flowing, gravity-driven volume of surfactant enters the lung, it is immediately drained leaving a predictable residual volume of surfactant that evenly coats the lungs. The rationale for the use of saline/surfactant lavage administration is three-fold. First, the dynamics of a fluid filled lung allow for easier more uniform lung expansion and consequently a more even distribution of surfactant. Secondly, lavaging allows for removal of alveolar debris including injurious cytokines, which inhibits surfactant function. Finally, and because of the first two, lavage administration allows for the use of lower doses of administered surfactant.

Whole lung lavage, using a combination of saline and surfactant, is efficacious in removing particulate matter from the lungs and ameliorating the Acute Lung Injury associated with aspiration and smoke inhalation. The major disadvantage of whole lung lavage is that it interferes with gas exchange during the lavage procedure. One may overcome this disadvantage by substituting PFC for saline.

When surfactant and PFC are mixed, the two rapidly separate to form an immiscible bilayer. This immiscible state is overcome with the addition of a small amount of methylprednisolone (a steroid) yielding a formulation called "Perfactant". It is believed that the steroid acts to form a reverse micelle with the hydrophilic head sequestering the surfactant and the hydrophobic tail forming bonds with the PFC (figure 2).

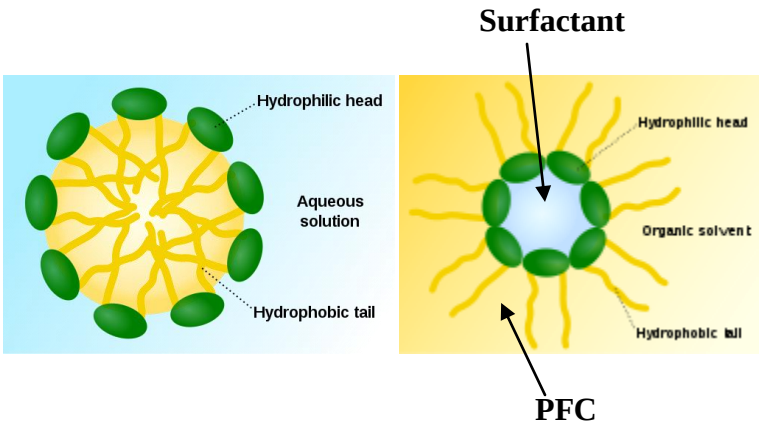


Figure 2

The schematic on the left is a typical micelle with its hydrophilic head oriented outwards. The head forms a bond with the aqueous solution, while the organic solvent is sequestered in the center by the hydrophobic tail. The schematic on the right represents a reverse micelle (Perfactant lavage). The blue area in the core represents surfactant sequestered by the hydrophilic heads. The hydrophobic tails form bonds with the PFC represented as the yellow outer area.

RELEVANCE:

Aspiration and inhalation induced ARDS is a frequent complication in both the military and civilian population. To date, there are few treatment options for ARDS beyond supportive measures. A gravity driven lavage procedure, performed with minimal equipment by medical personnel may reduce the morbidity and mortality associated with aspiration and inhalation events.

CURRENT DEVELOPMENT STATUS:

We were awarded a research grant by the US Air Force Office of the Surgeon General to characterize the physical properties of Perfactant Lavage. Our research thus far has focused on determining the lowest concentration of methylprednisolone required to achieve a miscible, homogenous solution.

Our initial trials with Perflurobron (Alliance Pharmeceutical Corporation, San Diego, CA), a perfluorocarbon derivative, failed to yield a miscible solution. We were, however, successful at achieving a homogenous solution using perfluorodecaline (F2 Chemicals LTD, Lancashire, Great Britian). It is believed that perfluorocarbon derivatives (perfluorocarbons with a functional group attached) may not be suitable for use in compounding Perfactant Lavage because the functional group interferes with the formation of reverse micelles. However, it is reasonable to assume that any liquid PFC would work. Examples of liquid PFCs suitable for use in formulating Perfactant Lavage include (but are not limited to): Perfluoro-chemical FC-43 (3M Specialty Chemical Div., Neuss, Germany), Perfluoro-chemical FC-75 (Acros Organics, Fisher Scientific, Fairlawn, New Jersey), Rimar-101 PFC liquid (Miteni Corp., Milan, Italy) and perfluoroperhydrophenanthrene (FluoroMed, L.P., Round Rock, Texas).

Our Perfactant lavage formulation uses the commercially available surfactant, Infasurf (Forest Pharmaceutical Inc., St Louis, Missouri). Because all commercially available pulmonary surfactants are similar in their composition, it is likely that these surfactants could be used to formulate Perfactant lavage. The commercially available forms of surfactant include (but are not limited to): Alveofact (Boehringer Ingelheim GmbH, Ingelheim am Rhein, Germany), Curosurf (Dey LP, Napa, California), Survanta (Abbott Laboratories, Abbott Park, Illinois), Exosurf (GlaxoSmithKline, Brentford, Middlesex, United Kingdom), Pumactant (Britannia, Pharmaceuticals LTD, Surrey, United Kingdom) and Surfaxin (Discovery Laboratories, Inc., Warrington, Pennsylvania).

Based on previous work (Huang *et al*, 2004), Infasurf containing 35 mg phospholipids/mL was diluted by perfluorodecalin (PFDC) instead of normal saline to administer a volume of 35 mL/kg containing 4 mg surfactant/mL. This translates into a dilution factor of 8.75, i.e., 1 mL Infasurf + 7.75 mL PFDC. Assuming that ~5 mL/kg (i.e., 1/7) will be retained in the lung after the drainage procedure, this calculates to a surfactant dose of (35 mL/kg) x (4 mg/mL) x (1/7) or 20 mg/kg.

Likewise, the dose of methylprednisolone (MPDS) diluted with the surfactant and PFDC solution was 150 mg/kg in order to achieve a retained dose of about 30 mg. The amount of MPDS to added is (150 mg/kg x 8.75 mL)/ (35 mL/kg) = 37.5 mg.

Thus, the optimal composition of the emulsion consists of (1 mL Infasurf + 7.75 mL PFDC + 37.5 mg MPDS). Dividing all the quantities by 5 in order to reduce the cost, this leads to (0.2 mL Infasurf + 1.55 mL PFDC + 7.5 mg MPDS).

METHODS:

Preparation of the emulsions - The emulsion was prepared by the vortex-mixing procedure at room temperature. The art of preparing the emulsion consisted in weighing a certain amount of MPDS with or without cholesterol in a plastic tube to which Infasurf was added immediately to generate a homogeneous solution by vortexing. Subsequently, PFDC was added in small aliquots while vortexing until a stable emulsion formed. Generally, an emulsion formed in less than 5 minutes during the vortex-mixing. Doing otherwise, a stable emulsion could not form.

Determination of the degree of stability of the emulsions – The physical stability of the emulsions was assessed by detection of phase separation as described elsewhere (Muderhwa *et al*, 1999) following incubation at 4, 25 and 37C. The data are expressed in % phase separation of the emulsion which is calculated as [(height of the oil phase)/(total height of the emulsion sample x initial fraction of oil in the emulsion)] x 100.

PRELIMINARY RESULTS**:**

Effect of MPDS concentration on the formation of the emulsions:

Several amounts of MPDS ranging from 2.5 mg to 120 mg (Table 1) were combined with 0.2 mL Infasurf and 1.55 mL PFDC in bottom-rounded test tubes and the resulting mixtures were vortexed as described in the Methods section to determine whether a stable emulsion will form in each case. It was determined experimentally that 1 mg of powder of methylprednisolone sodium succinate contained 0.29 mg of active methylprednisolone.

Table1: Effect of MPDS Concentration on the formation of emulsions.

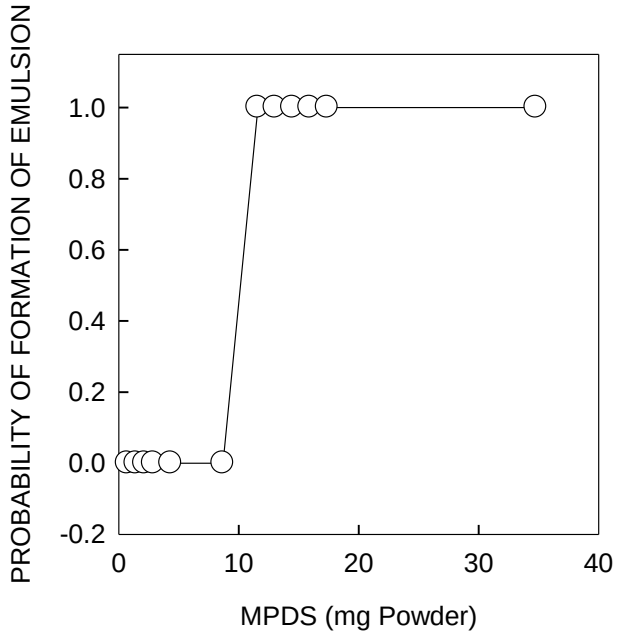
Methylprednisolone (MPDS)		**Formation of Emulsion**
Powder Quantity (mg)	*Active Compound Quantity*	
2.5	0.725	No
5	1.45	No
7.5	2.175	No
10	2.90	No
15	4.35	No
30	8.70	No
40	11.60	No

45	13.05	No
50	14.50	Yes
55	15.95	Yes
60	17.40	Yes
120	34.80	Yes

As shown in Table 1 and Figure 1, stable emulsions started to form in a narrow range of concentration between 13.05 and 14.50 mg of active MPDS (i.e., 45 and 50 mg of MPDS powder, respectively). Unfortunately, this concentration is higher than the required optimal dose of MPDS of 7.5 mg which was established in the Experimental Design section. Note that to draw the data shown in Figure 1, the YES for the formation of a stable emulsion was assigned the probability value of 1 and the NO formation stands for the probability value of 0.

~~Figure 1~~

Effect of MPDS Concentration on the Formation of PFDC Emulsions

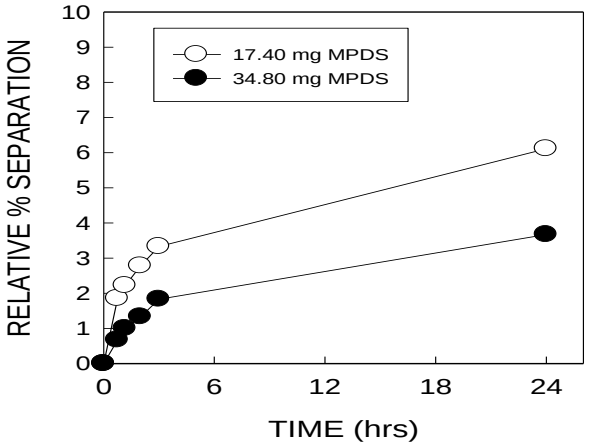


Effect of MPDS concentration on the degree of stability of the emulsions:

To determine the degree of stability of the emulsions, two emulsions containing 17.4 and 34.8 mg of active MPDS were prepared and their stability was monitored at room temperature (i.e., 24C). As Figure 2 shows, the degree of stability of the emulsions depended on the concentration

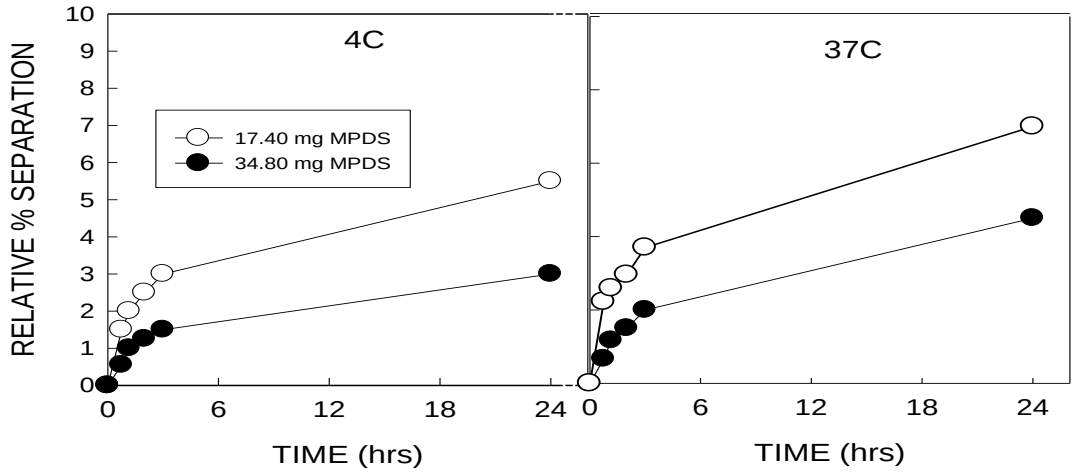
of MPDS. The rate of separation of the emulsions was high up to 3 hours of incubation reflecting a type of initial kinetic burst with the relative percent separations being 1.8% and 3.3% for emulsions formulated with 17.4 mg and 34.8 mg of active MPDS, respectively. Thereafter, the rate of separation progressed to an equilibrium state with the relative percent separations being 3.6% and 6.1% at 24 hours of incubation for emulsions formulated with 17.4 mg and 34.8 mg of active MPDS, respectively.

~~Figure 2~~
Effect of MPDS Concentration on the Stability of PFDC Emulsions



We have discovered that the separated emulsions described above could be "re-vortexed" after 24 hours of incubation yielding suitable emulsions that could be used for further characterization. Figure 3 shows below the degree of stability of such resulting emulsions following their incubation at 4C and 37C.

~~Figure 3~~
Effect of MPDS Concentration and Incubation Temperature on the Stability of PFDC Emulsions



These data suggest that the incubation temperature does not have any significant effect on the degree of stability of the emulsions (see Figure 2 vs. Figure 3). Following 3 hours of incubation at 4C, the relative percent separations were 1.5% and 3.0% for emulsions formulated with 17.4 mg and 34.8 mg of active MPDS, respectively. These numbers of relative percent separations increased steadily to 3.0% and 5.5% following 24 hours of incubation. Likewise, for emulsions incubated at 37C, the relative percent separations were 2.0% and 3.7% for emulsions formulated with 17.4 mg and 34.8 mg of active MPDS, respectively, at 3 hours of incubation. Also, these latter numbers of relative percent separations increased steadily to 4.5% and 7% following 24 hours of incubation.

Effect of cholesterol concentration on the formation of the emulsions:

As described earlier above, we have discovered that stable emulsions started to form in a narrow range of concentration between 13.05 and 14.50 mg of active MPDS (i.e., 45 and 50 mg of MPDS powder, respectively) (Table 1 and Figure 1). Unfortunately, this concentration is higher than the required optimal dose of MPDS of 7.5 mg which was established in the Experimental Design section. Since cholesterol (CHOL) and MPDS are structurally related (see Figure 4A and Figure 4B), we reasoned that part of the MPDS could be substituted for CHOL to formulate a stable emulsion.

Figure 4A: Cholesterol Molecular Structure

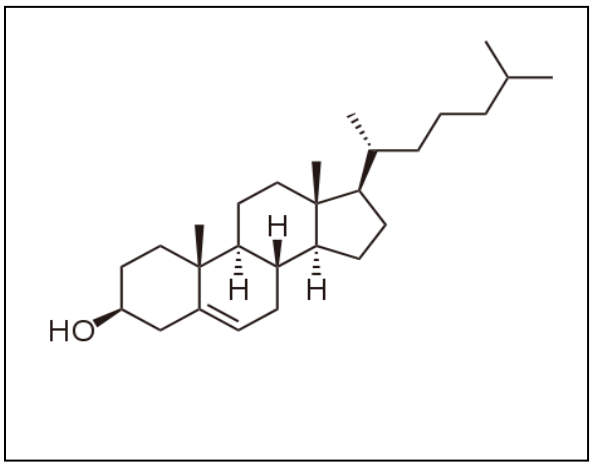
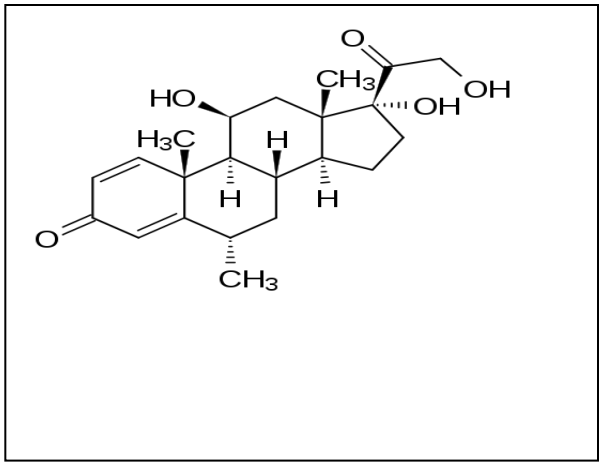


Figure 4B: MPDS Molecular Structure



We first conducted a trial experiment using 120 mg of cholesterol in the mixture with and without the optimal dose of MPDS of 7.5 mg. Table 2 shows that when MPDS was omitted from the mixture, the emulsion could be produced. This discovery indicates that MPDS was a *sine qua none* requirement for the formation of an emulsion in the presence of cholesterol.

Table 2: Effect of cholesterol with and without MPDS on the formation of the emulsions.

Composition of Emulsion				**Formation of Emulsion**
MPDS (mg)	Cholesterol (mg)	Infasurf (mL)	PFDC (mL)	
0	120	0.20	1.55	NO

7.50	120	0.20	1.55	YES

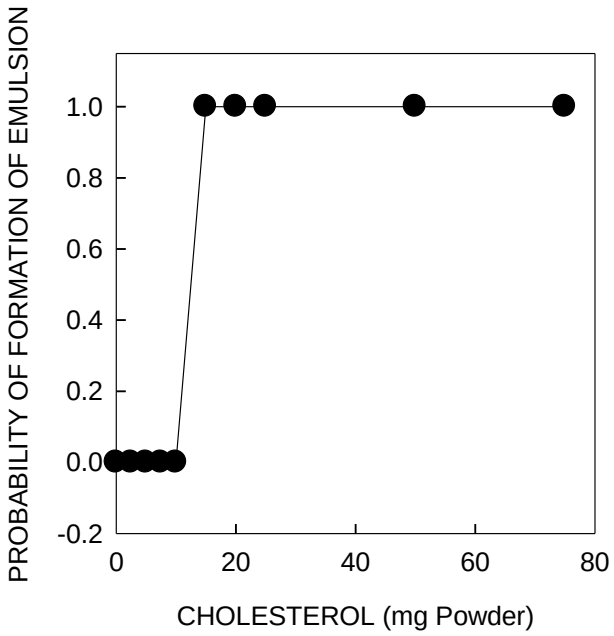
In the next experiments, several amounts of cholesterol ranging from 0 mg to 75 mg (Table 3) were combined with 7.5 mg MPDS, 0.2 mL Infasurf and 1.55 mL PFDC in bottom-rounded test tubes and the resulting mixtures were vortexed as described in the Methods section to determine the range of concentration in which a stable emulsion will form.

Table 3: Effect of various concentrations of cholesterol on the formation of emulsions.

Cholesterol (mg)	**Formation of Emulsion**
0	NO
2.5	NO
5	NO
7.5	NO
10	NO
15	YES
20	YES
25	YES
50	YES
75	YES

As shown in Table 3 and Figure 51, stable emulsions started to form in a narrow range of concentration between 10 mg and 15 mg of cholesterol. As explained earlier above, to draw the data shown in Figure 5, the YES for the formation of a stable emulsion was assigned the probability value of 1 and the NO formation stands for the probability value of 0.

~~Figure 5~~
Effect of Cholesterol Concentration on the Formation of PFDC Emulsions



Effect of Solec F concentration on the formation of the emulsions:

In this set of experiments, several amounts of Solec F, a powdered egg lecithin with a hydrophilic-lipophilic balance (HLB) of 7 (Solae, Inc, St Louis, MO) ranging from 0 mg to 25 mg (Table 4) were combined with 7.5 mg MPDS, 15 mg cholesterol, 0.2 mL Infasurf and 1.55 mL PFDC in bottom-rounded test tubes and the resulting mixtures were vortexed as described in the Methods section to determine the range of concentration in which a stable emulsion will form. As the data of Table 4 and Figure 6 show below, emulsion was produced when Solec F was not part of the composition of the emulsion. This also occurred when Solec F was added in the range of concentration of 15-25 mg. Interestingly, no emulsion was formed for Solec F concentrations of 5 mg and 10 mg. It is possible that Solec F is interfering with the effect of one or more components of the emulsion until enough of it is added to the emulsion mixture to suppress this negative effect.

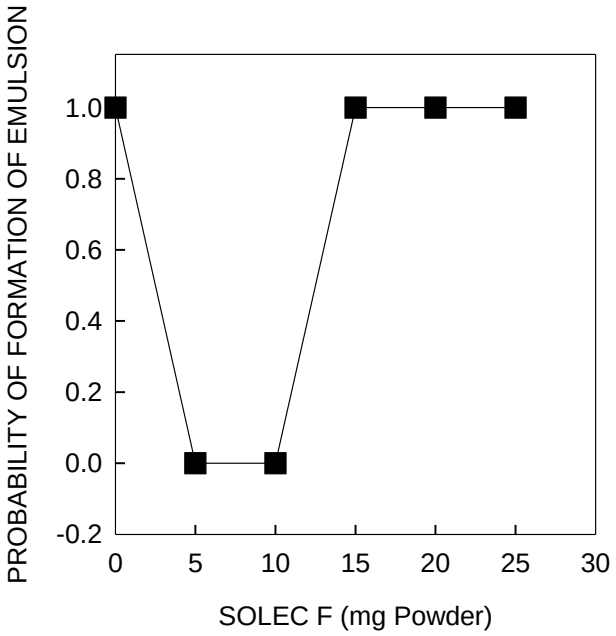
Table 4: Effect of various concentrations of Solec F on the formation of the emulsions.

Solec F (mg)	**Formation of Emulsion**
0	YES
5	NO

10	NO
15	YES
20	YES
25	YES

~~Figure 6~~

Effect of Solec F Concentration on the Formation of PFDC Emulsions



FUTURE EFFORT:

1. Determine the degree of stability of the emulsions formulated with the addition cholesterol.
2. Effect of PFDC concentration on the formation and stability characteristics of the MPDS-PFDC-KL4-cholesterol (CHOL) emulsions.
3. Viscosity properties of the MPDS-PFDC-KL4-cholesterol (CHOL) emulsions.
4. Size distribution of the MPDS-PFDC-KL4-cholesterol (CHOL) emulsions.
5. Oxygen solubility in and oxygen transfer from the MPDS-PFDC-KL4-cholesterol (CHOL) emulsions.
6. Lipid profile of the MPDS-PFDC-KL4-cholesterol (CHOL) emulsions
7. Surface pressure and surface tension characteristics of the MPDS-PFDC-KL4-cholesterol (CHOL) emulsions to determine their biophysical properties and correlation activity to natural lung surfactants (NLSs).
8. Inclusion of non-ionic polymers such as dextran, polyethylene glycol and Pluronic F-68 to the emulsions to prevent their inactivation against plasma leakage.

TERMINATION OF PROJECT**:**

Due to a 12-month deployment to Afghanistan and two assignment changes, I have been unable to devote the time nor secure the technical assistance necessary to further this research. I am seeking a new assignment at the U.S. Army Institute of Surgical Research beginning September 2015. If selected, I plan on resuming this research; however, because assignments are not guaranteed, I believe it best to close out this project. I wish to thank the U.S. Air Force for their generous support in this endeavor.